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Evaluation of Wound Healing Potential of ethanolic extract of Tridax Procumbens L. in clinical models

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ABSTRACT

Wound healing is a complicated process in which the skin or the afflicted organ heals itself after an injury. In numerous animal models, scientific validation for the use of most of these botanical parts and species in the management and treatment of prenatal and postnatal complications has been reported. As a result of its bioactivity, biocompatibility, osteoconductivity, structural and chemical similarity to bone and teeth, host tissue bonding ability, and absence of inflammatory characteristic, it has the capacity to regenerate tissue. Our study aimed to identify the wound healing property of Tridax procumbens in Clinical models. In this study we have identified the various phytochemical activities such as cytotoxic effect, anti inflammatory, anti microbial, free radical scavenging and wound healing activity of tridax procumbens. It was found that Tridax procumbens accelerated the healing process by enhancing the epithelial regeneration of wounds. The potent activity of the herbal formulation can be attributed to the phyto-constituents present in the formulation enhance the wound healing effect. Additional research is necessary to understand both the target-based mechanism of action and other pharmacological effects

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INTRODUCTION

Wound healing is a complicated process in which the skin or the afflicted organ heals itself after an injury. Under normal circumstances, the epidermis (outermost layer) and dermis (inner or deeper layer) of the skin are in steady-state symmetry and constitute a protective barrier against the external environment (1). Growth factors function through autocrine, paracrine, and endocrine signaling systems to initiate wound healing. Aside from them, there are a slew of growth factors that help wound healing through a variety of processes (2). Connective tissue proliferation is stimulated by platelet-derived growth factor (PDGF), cutaneous tissue proliferation is stimulated by epidermal growth factor (EGF), and fibroblast proliferation is stimulated by fibroblast growth factor (FGF) (3). An epidermal wound is a pathologic condition characterized as a break in the skin's integrity. Blood loss, discomfort, edema, inflammation, and loss of functionality are all linked to a high level of morbidity (4).

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Hydroxyapatite,
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wound healing,
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Scientific studies have revealed how wounds are characterized by fibroblast proliferation and migration, epithelial and endothelial proliferation, connective tissue deposition, angiogenesis, re-epithelization, and wound contraction (5). Proper healing of wounds refers to repair and restoration of tissues. It's debatable if systemic medications can speed up healing in someone who is nutritionally and endocrinologically healthy. In such circumstances, basic wound healing principles such as minimizing tissue damage, debriding nonviable tissue, increasing tissue perfusion and oxygenation, appropriate nutrition, and a moist wound healing environment is reliable(6). Major inorganic mineral hydroxyapatite, which promotes cell adhesion and proliferation, is found in bone and teeth.

Tridax procumbens (T. procumbens) Linn. is a tropical, subtropical, and moderate temperate plant species which is found to be reliable in ayurvedic medicines for liver diseases, boils, open wounds, cuts as an anticoagulant, antifungal, and disinfectant (7). Flavonoids, alkaloids, carotenoids, hydroxycinnamates, lignans, benzoic acid derivatives, phytosterols, and tannins are all known to be present in the plant (8). Many researches have done to prove the various pharmacological properties, including hepatoprotective activity, anti-inflammatory activity, wound repair, antidiabetic activity, hypotensive effect, immunomodulating property, bronchial coughs, constipation, diarrhea, and to prevent hair loss by stimulating hair growth. It also exhibits antimicrobial activity against both gram-positive and gram-negative bacteria. Leaf juice is used to heal wounds in the dead space (9). Seeds are used to prevent the variety of bleeding. As an immunosuppressant, aqueous extract of the entire part is employed. Even when formulated in a mineral base, dry extract demonstrated antibacterial activity (10). Calcium carbonate, phosphates, and reactive amine groups were combined to form hydroxyapatite-based nanocomposites through hydrolysis, cross-linking, and functionalization. Due to its excellent biocompatibility and appropriate angiogenic capacity, hydroxyapatite (HAP) has a significant potential for wound healing (11).

This study reveals details on how herbal medicines can help with a variety of skin disorders as well as wound healing. Traditional medicine has been encouraged by the World Health Organization (WHO) since it is less expensive, more accessible, and comprehensive, especially in developing countries. The synthesis of natural ingredients in the herb- or plant-derived products often has curative effects. These characteristics considerably expand a range of potential therapeutic development prospects; yet, several safety-related worries have surfaced. (12–20). Our study aimed to identify the wound healing property of Tridax procumbens in Clinical models.

MATERIALS AND METHODS

Study setting

The Nano Research lab at Saveetha Dental College and Hospital, Chennai is where the current in vitro investigation was carried out. Tridax procumbens plant extract had been available in powdered form that could be specially ordered from Madhavaram Botanical Garden, Chennai. The readily available plant snippets are a benefit in reducing physical labor and time. The ethical approval was done by our scientific review board committee with ethical number IHEC/SDC/FACULTY/22/GPATH/454. Six adult zebrafish (3 male, 3 females) in standard, control and test groups were included in the study group. Similar grouping was done with Healthy wistar rats as mentioned earlier. Random sampling was carried out in this study. Validation of the procedure was given by the Senior Nanoresearch guide.

Inclusion criteria include healthy adult rat and zebrafish models.. Exclusion criteria include steatotic, unhealthy, underweighted rats and zebrafish which had died during treatment time period were not taken under consideration.

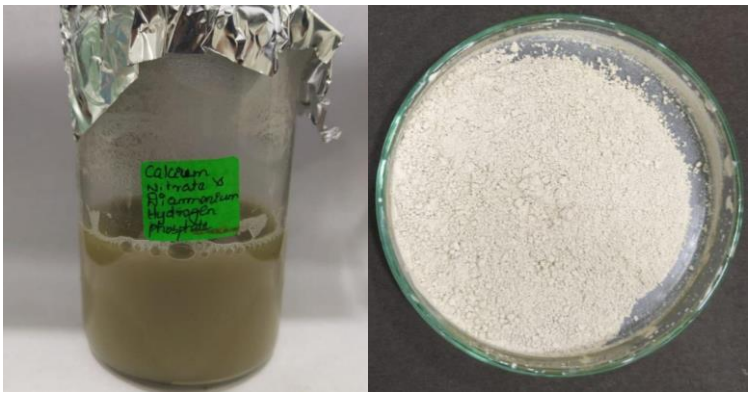

Figure 1: Preparation of Tridax procumbans and Preparation of Hydroxyapatite nanoparticles after mixing Calcium nitrate, diammonium hydrogen phosphate and ammonia (for pH maintenance at 8).

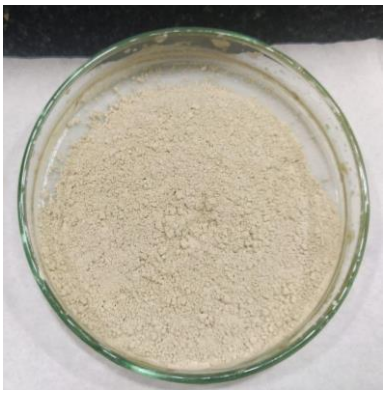

Figure 2 : Preparation of Tridax Hydroxyapatite nanoparticles.

Preparation of plant extract

Tridax procumbens were weighed and added to 100 mL of distilled water. The mixture was then heated at 75°C for 20 minutes to create the extract. After filtration, the extract was reboiled at 75°C. For 10 minutes, the leaf extract was centrifuged at 8000 rpm. Followed by centrifugation, the Tridax precipitate was transferred to a petri dish and preserved in a hot air oven at 100°C for 24 hours.

Hydroxyapatite extract preparation

11.8 grams of calcium nitrate and 3.96 grams of di-ammonium hydrogen phosphate were used to make hydroxyapatite crystals in 50 mL of deionized water. Overnight, the solution was placed in a magnetic stirrer. Calcium nitrate and diammonium hydrogen phosphate solution was treated with ammonia until the pH reached 8. As a control, distilled water was placed in a centrifuge tube along with the hydroxyapatite precipitate, and the tube was centrifuged at 8000 rpm for 10 minutes. The petri dish with white precipitate is then placed in a hot air oven set at 100°C for 24 hours. The hydroxyapatite is dried, ground into a fine powder, and stored.

Tridax hydroxyapatite extract preparation

Hydroxyapatite is mixed with a 65:35 ratio of Tridax procumbens leaf extract overnight in a magnetic stirrer. Using UV spectrophotometry, successive absorbance measurements are used to determine nanoparticle production. To 5mL of distilled water, 100mg of Tridax procumbans and HAP crystals were measured and added. Nano agglomerates are broken up by separating nanoparticles from liquids using a digital ultrasonic cleanser for 15 minutes at 50°C. HAP and tridax HAP were divided into various concentrations of 20µL, 40µL, 60µL, 80µL, 100µL.

Wound creation in clinical models

Healthy wistar rats of (150-200g) and zebra fishes (20g) were anesthetized using tricaine (100 mg/l). Incision was done less than 2 mm. Zebrafish caudal fins were cut 4-5mm from the back end with a sterile scalpel. Tridax hydroxyapatite was given to one of the three experimental groups, hydroxyapatite was given to another, and hydrogel was given to the third group as the control. The Caudal fin growth and wound closure of wistar rat were measured. Histopathological analysis of Zebra fish and wistar rat were done on 5 th and 14 th day of the samples.

Figure 3: Tridax Hydroxyapatite, Hydroxyapatite, Hydrogel (Standard) and control group of Zebrafish and wistar rats.

Evaluation

Three rats and four fishes each in the groups T(HAP), HAP, Standard, and control. Toxicology test was carried out to determine the therapeutic dose of the extract, lower dose was considered for further studies, rats and zebrafish were divided into control and standard group. Homed under 23°C for 12 hours of light and dark cycle. Extract was applied twice daily for two weeks.

Cytotoxic effect (Brine Shrimp Lethality assay)

2 g of iodine-free salt was mixed with 200 µL of distilled water. 10-12 µl of saline was added to a 6-well ELISA plate. Each well acquired 10 nauplii introduced sequentially (20 µl, 40 µl, 60 µl, 80 ml and 100 µl). Subsequently, nanoparticles of HAP and TP-HAP were imported depending on the concentration. Plates were incubated for 24 hours. ELISA plates were examined after 24 hours to determine the number of viable nauplii present. This number was calculated using the following formula: number of dead nauplii/number of dead nauplii+number of live nauplii×100.

Anti-inflammatory activity

Bovine serum albumin assay (albumin denaturation assay)

To test the anti-inflammatory potential of TP-HAP based gel, Muzushima and Kabayashi suggested the following convention, with some modifications (Pratik Das et al.,2019). 10 µL, 20 µL, 30 µL, 40 µL, and 50 µL of Tridax procumbans extract were combined with 1 percent bovine serum albumin, and the pH of the mixture was raised to 6.3 by adding a little amount of 1N hydrochloric acid. After being incubated at ambient temperature for 20 minutes, these samples were heated to 55°C in a water bath for 30 minutes. After the samples were cooled, a spectrophotometric calculation of the absorbance at 660 nm was made. The standard was sodium diclofenac. DMSO is the controlling agent.

The following equation was used to calculate the percentage of protein denaturation:

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Antimicrobial activity

Using Mueller-Hinton agar, the zone of inhibition for each nanoparticle's antibacterial activity against strains of Staphylococcus aureus and Pseudomonas was identified. Mueller Hinton Agar needed to be prepared and sterilized for 45 minutes at 120 pounds. The media was placed in sterile plates and allowed to harden. After the wells were cut with the well cutter, the test organisms were swabbed. The plates were filled with various nanoparticle concentrations, and they were then incubated for 24 hours at 37°C. After the incubation time, the zone of inhibition was evaluated.

Hydroxyl radical scavenging assay

With a few minor modifications, the test was carried out according to the Halliwell technique [Halliwell et al., 1987]. Each solution was made from scratch using 1.0 mL of the reaction mixture, which included 200 mL of 200 M $FeCl_3$, 1.04 M EDTA (1:1 v/v), 100 mL H_2O_2 (1.0 M), and 100 mL ascorbic acid. The reaction mixture also contained 100 L of 28 M 2-deoxy-2-ribose, various concentrations of Tridax procumbans (10 to 50 l), and 100 L of 200 M Fe (1.0mM). The degree of deoxyribose breakdown was determined by the TBA reaction during an hour-long incubation time at 37°C. At 532 nm or so, compare the absorbance to a blank solution. A positive control was vitamin E.

RESULTS

Activity of reaction mixtures at five different concentrations of 10µL, 20 µL, 30µL, 40 µL, and 50µL was evaluated. The nauplii were subjected to various concentrations of Nano Hydroxyapatite-based green formulation of different concentrations (10µL, 20µL, 30µL, 40µL, and 50µL) and kept undisturbed for a period of 48 hours. For the purpose of evaluating the amount of cytotoxicity, the number of live nauplii that were still alive after 24 hours in each well with extracts of different concentrations was noted. The plant extract had a strong anti-inflammatory activity of 88.3%, which is near to the standard value percentage of 91.1%, when mediated by hydroxyapatite nanoparticles at a concentration of 50µL. Antimicrobial activity of Tridax HAP and HAP were found to have in vitro antibacterial activity against S.aureus and Pseudomonas, and the zone of inhibition was quantified. Disk diffusion test was used to quantitatively measure the nanoparticles at additions of 25µL , 50µL, and 100µL of Tridax HAP and HAP in individual discs. Maximum HAP's zone of inhibition against S. aureus and Pseudomonas was 15mm and 9mm respectively. Maximum TP-HAP was found to be 18mm and 9mm against S. aureus and Pseudomonas, respectively. The effectiveness of nanoparticles as antibacterial medicinal agents is so obvious. In free radical scavenging activity the percentage of inhibition at various doses in comparison to the standard is used to depict the antioxidant activity of Tridax procumbens herbal formulation in the above graph. Maximum inhibition was observed in S.aureus. The results indicated that TP-HAP has stronger and more active scavenging than HAP. Wound healing activity of the zebrafish model is established by fin regeneration on day six and twelveth. Histology of untreated wounded zebrafish models revealed normal epithelium, with caudal fin growth of 3mm on the sixth day and 6mm on the twelfth day, and histology of wistar rats revealed skin epithelization.

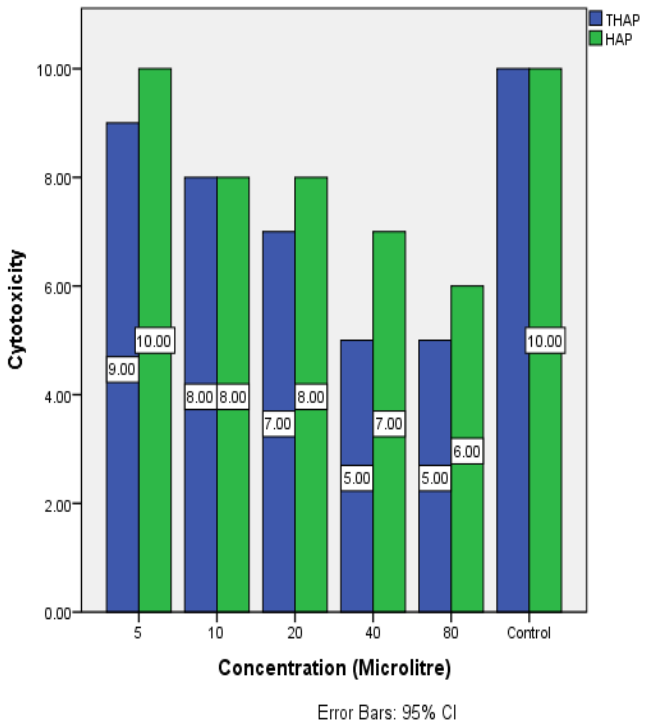

Figure 4 : The cytotoxic effect of T(HAP) and HAP is shown in the above graph under varying concentrations in the x-axis and amount of cytotoxic effect in the y-axis.

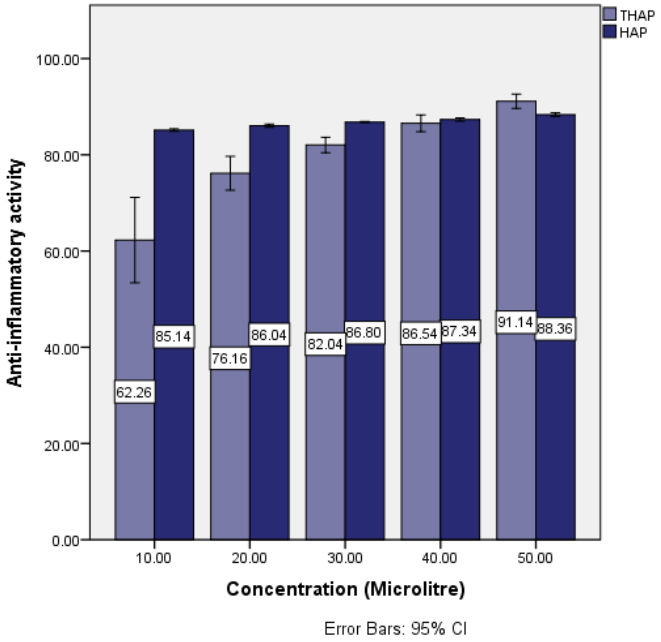

Figure 5: The anti-inflammatory activity of T(HAP) and HAP is shown in the above graph under varying concentrations (µL) in the x-axis and amount of anti-inflammatory in the y-axis.

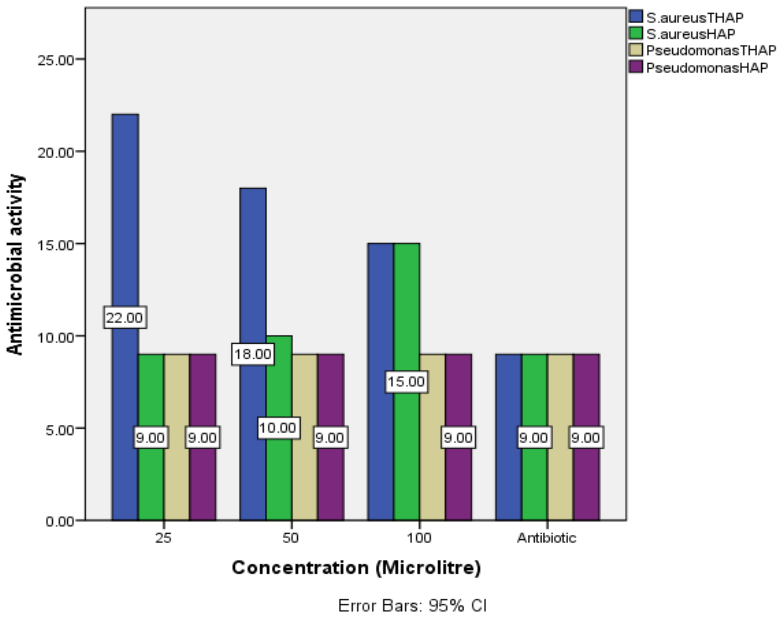

Figure 6: The antimicrobial activity of T(HAP) and HAP is shown in the above graph under varying concentration(l) in the X axis and amount of inhibition in the Y axis(mm).

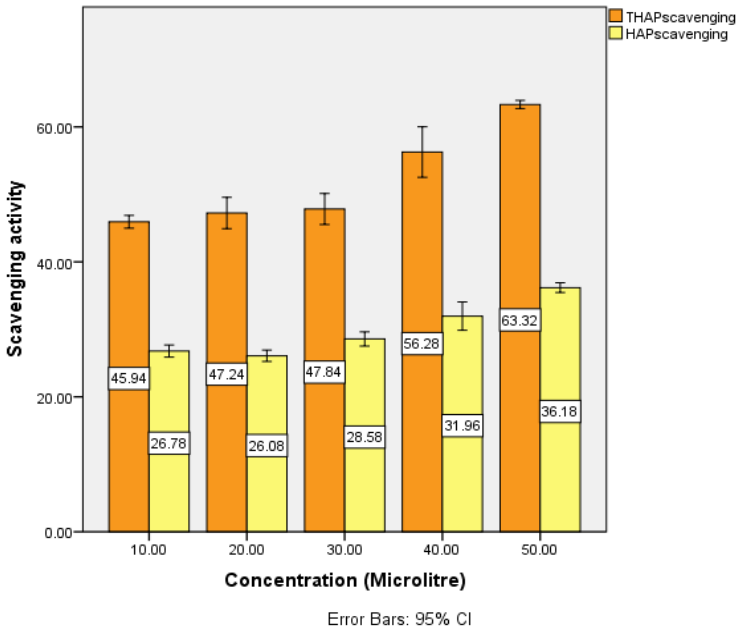

Figure 7: The positive free radical scavenging activity of T(HAP) and HAP is shown in the above graph under varying concentrations (l) in the x-axis and amount of scavenging activity in the y-axis.

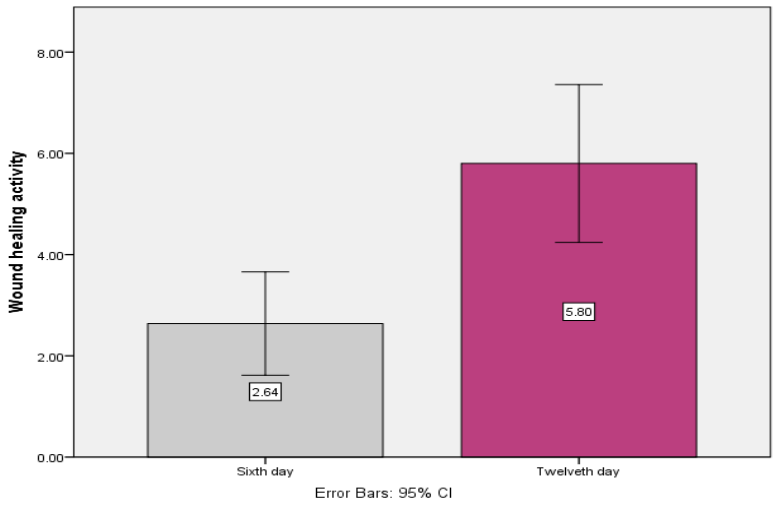

Figure 8: The wound healing activity of T(HAP) and HAP is shown in the above graph under varying concentrations (l) in the x-axis and amount of wound healing activity (mm) in the y-axis. On the 6th day T(HAP) showed maximal caudal fin growth of zebra fish of 3mm and 6mm on the 12th day.

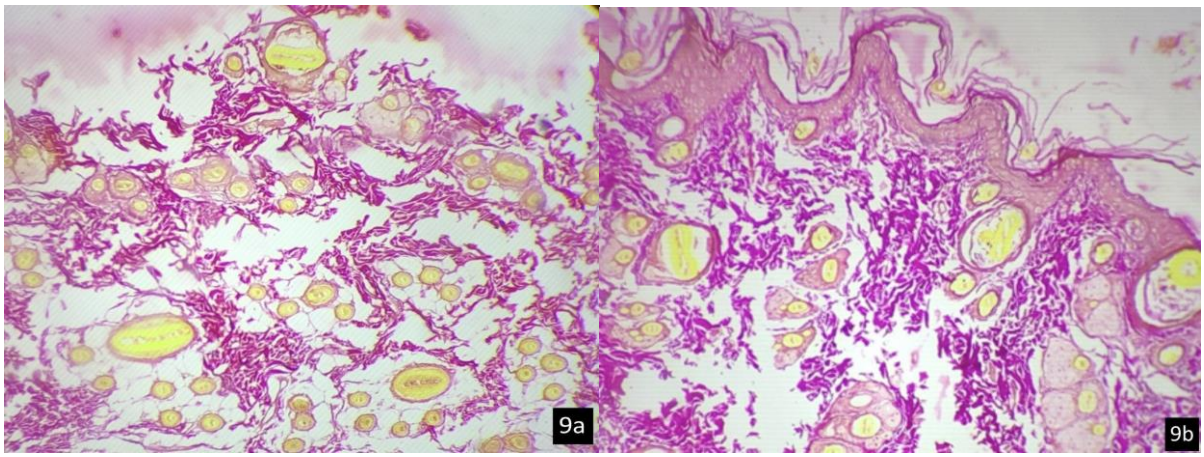

Figure 9: Histopathological images representing regeneration of tissues in wistar rats. Serial sections were stained with Hematoxylin & eosin. Figure 2a shows the untreated wounds and figure 2b shows treated wounds with epithelial regeneration, fibroblast proliferation and neovascularization in the connective tissue respectively.

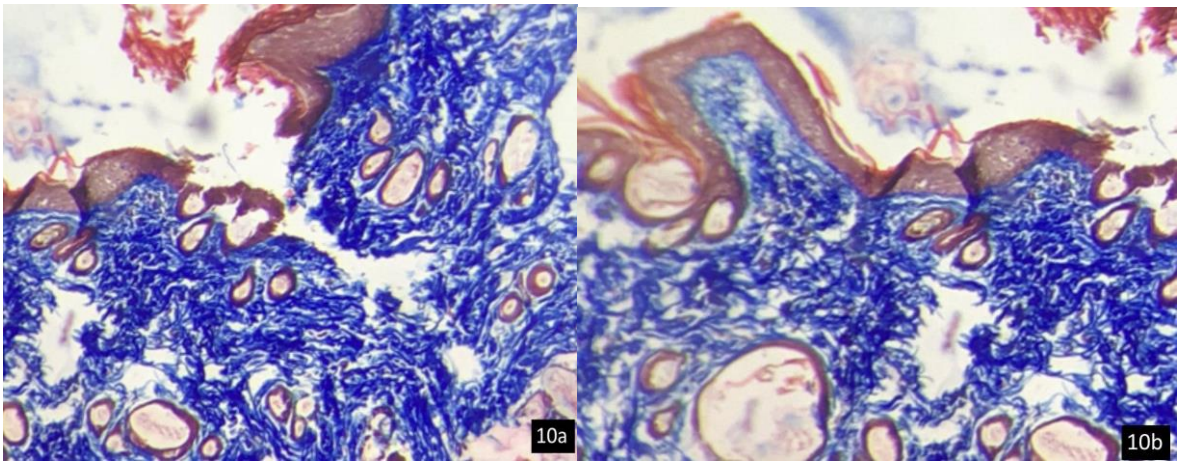

Figure 10: Histopathological images representing regeneration of tissues in wistar rats. Serial sections were stained with Masson Trichrome Figure 3a shows the untreated wounds and figure 3b shows treated with epithelial regeneration and fibroblast proliferation respectively.

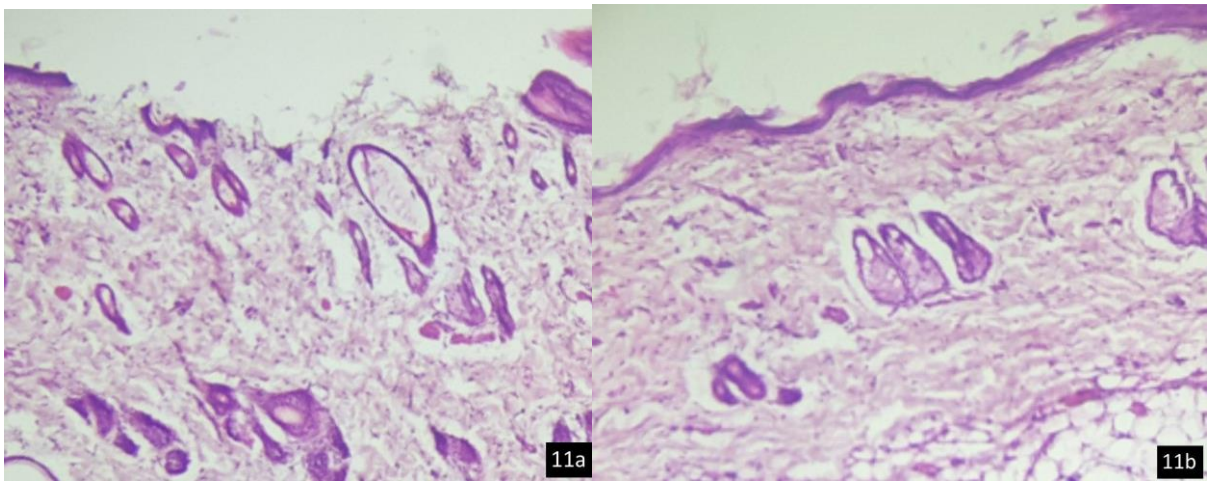

Figure 11: Histopathological images representing regeneration of tissues in wistar rats. Serial sections were stained with van gieson and evaluated microscopically for fibroblast proliferation, neovascularization and epithelial regeneration. Figure 1a shows the untreated wounds and figure 1b shows treated wounds respectively.

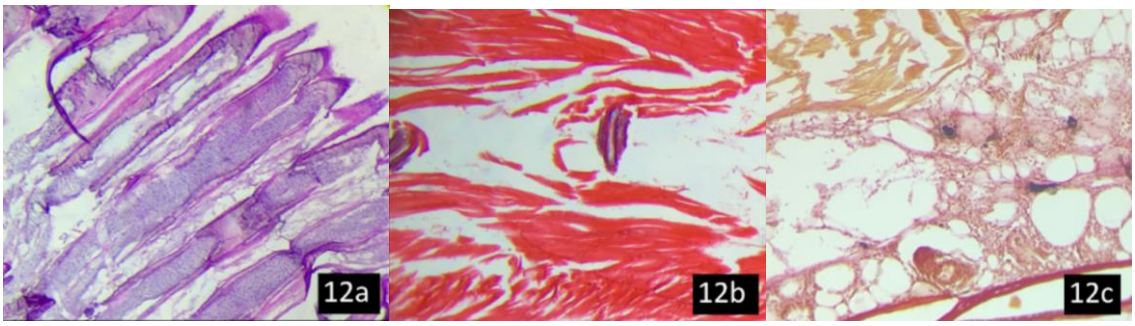

Figure 12: Histopathological images representing regeneration of tissues in Zebrafish. Serial sections were stained with 12a. Hematoxylin & eosin E, 12b. Masson trichrome and 12c. Van gieson stain respectively and evaluated microscopically for fibroblast proliferation, neovascularization and epithelial regeneration.

DISCUSSION

Herbal medicines are thought to be safer than allopathic drugs since allopathic medicines have adverse effects. The perception that plants are safe, trustworthy, and have fewer side effects has generated significant interest in medications produced from plants. The significant results in the present study shows that Tridax procumbens has significant cytotoxicity activity, antiinflammatory, antimicrobial, free radical scavenging and wound healing properties at higher concentration (21,22). Aqueous extract was also effective in increasing wound contraction but to a lesser degree than ethanolic extract. Wound healing involves a complex interaction between epidermal, dermal, extracellular matrix, controlled angiogenesis and plasma-derived proteins all coordinated by an array of cytokines and growth factors (23). The mode of anti-inflammatory activity of phenolic compounds is believed to be associated with their scavenging capacity through the donation of hydrogen atoms. Previous studies discovered that the whole plant of Tridax has antimicrobial activity against various species of bacteria. Because of this property, it is used to protect against human dermal infection and may aid in faster wound healing (24).

Phenolic compounds play a role in defensive mechanisms and have been related to better health. Phenolic substances function as antioxidant properties and guard against microbial infections, helping to prevent infectious and chronic diseases (25). Polyphenols called flavonoids have been found to be linked to a reduced risk of severe degenerative illnesses. It has antioxidant qualities that could aid to lessen cellular oxidative stress. Tannins are phenolic compounds that exist naturally and have medicinal and pharmacological effects. Studies have shown that tannins have a significant potential for healing or at least minimizing a number of diseases and syndromes .

According to previous research, the majority of anti-inflammatory drug efficacy occurs either by maintaining the lysosomal membrane or by inhibiting the release of these enzymes, which is one of the inflammatory responses (26). Many biomaterials have been created using hydroxyapatite. It demonstrated stronger encouraging effects on re-epithelialization, matrix formation, and wound healing (27). HAP also showed tissue growth within the uncoated control implants was substantially delayed and primarily occurred on their surface, whereas a severe foreign body reaction and inflammatory reaction were seen by day three post-operation

(28). Thus, there is a significant need for greater scientific and clinical comprehension of the processes underlying wound healing. Here, we go through the cellular foundation of tissue restoration and talk about how a better knowledge of the pathophysiology of wounds might guide the creation of future effective wound treatments.

Tridax procumbens contains a variety of beneficial constituents, including flavone glycoside, chromone glycoside, bithiophenes, glycosides (Procumbenetin), sterols, terpenoids, lipids, and polysaccharides, all of which have significant pharmacological properties such as hypotensive, hepatoprotective, anti-inflammatory, and antidiarrheal (26,29). It has wound-healing, antiparasitic, leishmanicidal, and hair-growth promoting properties in accordance with the previous research. Aside from that, it has antimicrobial and immunomodulatory properties, which provide a foundation for future research (30).

Also it was found that Tridax has traditionally been used to treat wounds in India. There is no mention of different dosage forms of Tridax in ayurvedic texts. The experimental trial of different dosage forms of Tridax on wound healing in albino rats reveals that Tridax has a highly significant improvement in wound healing, which include wound contraction and epithelialization, with the exception of the oil, which is not efficient in decreasing time for epithelialization (31). Future studies should concentrate on the relationship between specific phytochemicals and their effects on various ailments. Other areas that have yet to be thoroughly investigated include, but are not limited to, extraction yield, concentration, and physiological activity of these phytochemicals. Discoveries in these areas would provide important information that the medical community can use for preventative medicine and/or the development of new drugs. Many significant features of T. procumbens have yet to be discovered.

CONCLUSION

Tridax procumbens accelerated the healing process by enhancing the epithelial regeneration of wounds. The potent activity of the herbal formulation can be attributed to the phyto-constituents present in the formulation enhance the wound healing effect. Additional research is necessary to understand both the target-based mechanism of action and other pharmacological effects.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The authors would like to declare no conflict of interest in the present study.

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